IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Andrew Dames et al.

Serial No. 09/787,195

Filed: September 17, 1999

For: Bio-Assay Technique

Examiner: Lyle A. Alexander

Group Art Unit: 1743

SUPPLEMENTAL DECLARATION

I, Peter Swarbrick, hereby declare that:

1. I am the same Peter Swarbrick who signed a declaration dated 22 December 2005 in respect of US patent application no. 09/787,195 entitled "Bio-Assay Technique, (hereinafter the '195 application).

2. A driving force behind the invention of the '195 application is to achieve significant economies in terms of reduced labour, overheads and reagent costs, combined with significant savings in time to result, for a given panel of assays compared with e.g. conventionally performed enzyme-linked immunosorbent assays (ELISA) in which samples are coated to the surfaces of the wells of a microtiter plate. Time to result is particularly important in medical diagnosis. The support claimed in the '195 application provides results with sensitivities equivalent to conventionally performed ELISA assays (the testing method in common use by most commercial users), whilst at the same time by enabling multiple assay tests per well it generates orders of magnitude cost and time savings. Typically, the support claimed in the '195 application uses only 15% of the capture reagents necessary to perform an equivalent ELISA. A further advantage of enabling multiple assay tests per well is that many more data points are generated, which can be particularly important in research applications, e.g. drug discovery and clinical trials. Using ELISA assays, scientists or clinical technicians would typically need to perform tests in duplicate merely to ensure repeatability and validity of the tests results.

3. US 5129974 (Aurenius) describes barcoded particles with external dimensions of 1 x 1 x 0.1 mm for use as labels for integrated circuits. These particles are many orders of magnitude

larger than the claimed support, which has a largest external dimension of less than 100 microns (0.1 mm). A typical embodiment of the claimed support might have external dimensions of 0.1 x 0.01 x 0.001 mm. Using "Aurenius" particles in bioassays, even if practically achievable (see paragraph 8 below for further discussion of this), would dramatically limit both the number of particles that could occupy a given space, which would severely limit the number of tests which could be performed. "Aurenius" particles would also consume far greater amounts of sample and reagents compared to embodiments of the claimed support. This is demonstrated in the following images.

4. Figure 1, below, shows an image at ~15X magnification of a single standard ELISA microtiter plate well containing particles. Distortion in the image is caused by the mosaicing together of several smaller images. The well is approximately 7mm in diameter. Using conventional ELISA techniques in which a sample is coated to the surface of a well would generate a single data point per well. The large plate-like particles shown in the image (black rectangles, each with three holes), have external dimensions of 0.3 x 0.6 mm. This is in fact *smaller* than the "Aurenius" particles, which in terms of surface area would be about six times bigger, but nonetheless the large particles serve to demonstrate the effect of using particles larger than the claimed support. There are 40 large particles in the image, but only 12 are physically isolated from the others. As physical isolation is necessary to achieve particle identification via the barcodes (i.e. the three holes) and for the unambiguous reading of fluorescence emissions from the particles (to avoid misreads and signal transfers from one particle to another), there are effectively therefore just 12 usable particles in the image.

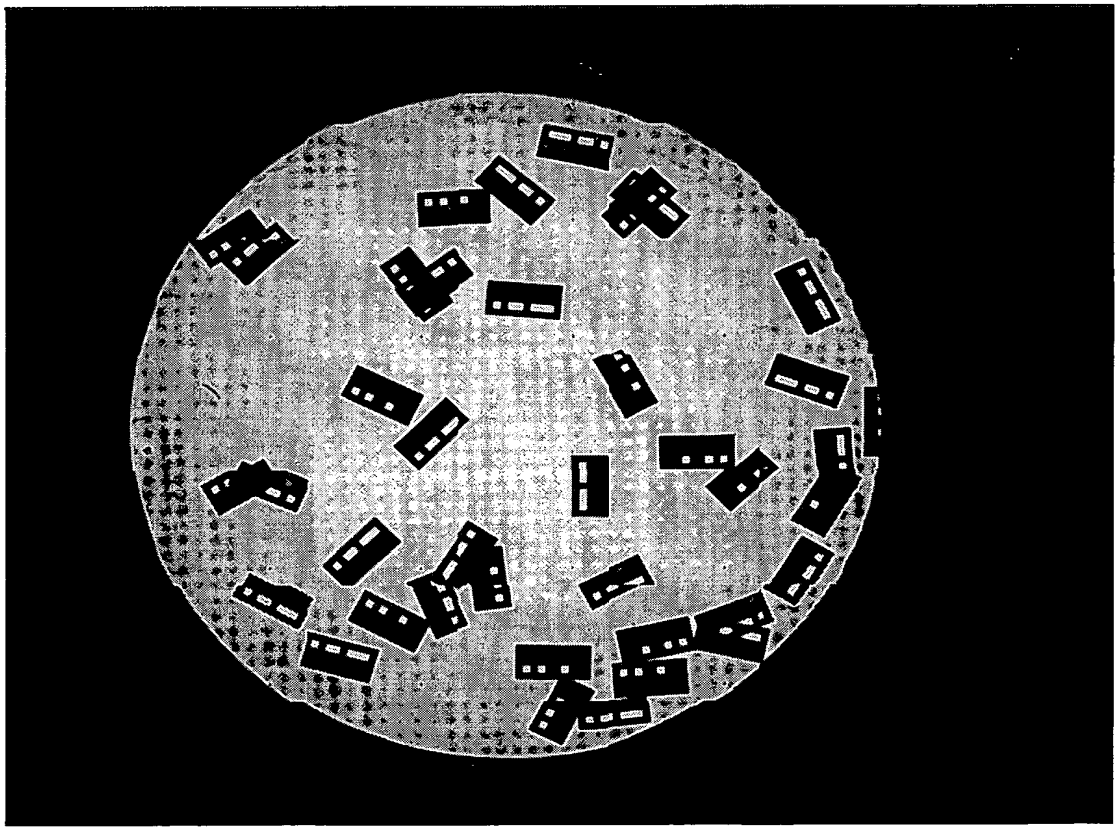

Figure 1

5. Figure 2, below, shows another 7mm diameter standard well at ~15X magnification, but now containing both large particles and 0.1 x 0.01 x 0.001 mm sized small particles embodying the claimed support. It is clear that orders of magnitude more of the small particles (i) can be contained in the standard well and (ii) are physically isolated from each other and therefore capable of being identified and read.

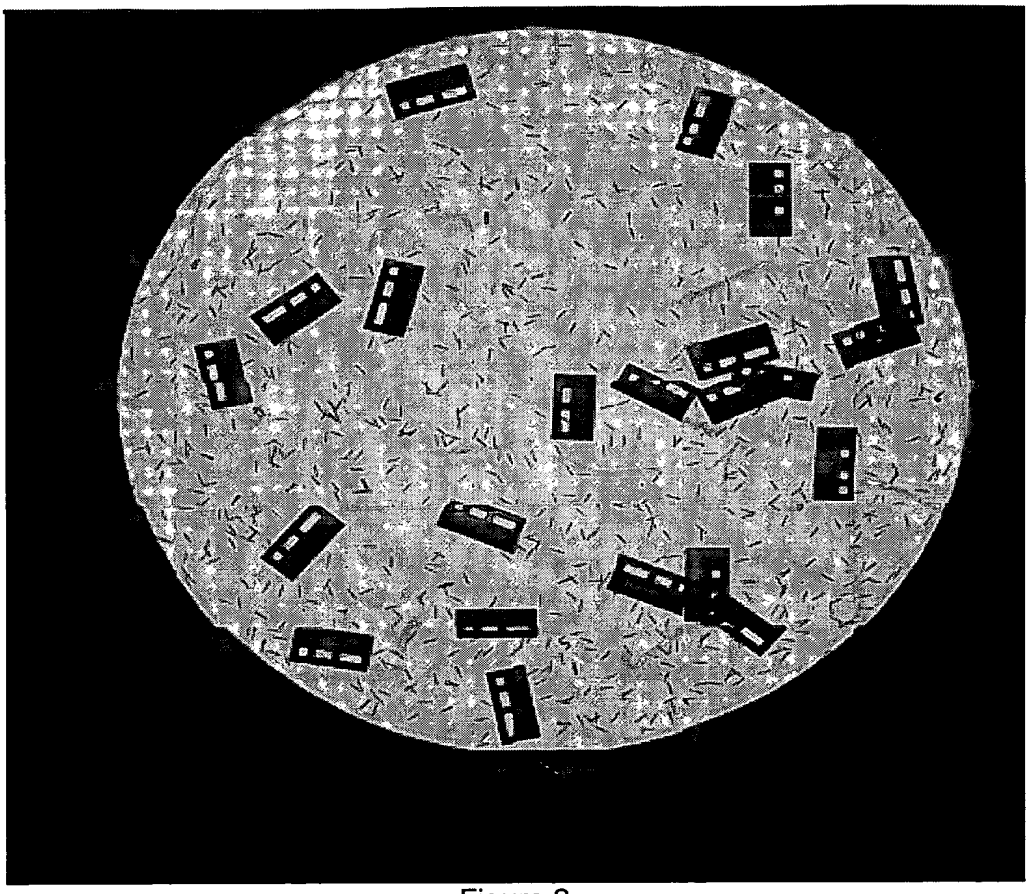

Figure 2

6. Figure 3, below, is a higher magnification image at ~150X magnification of both types of particles and demonstrates the enormous difference in relative size.

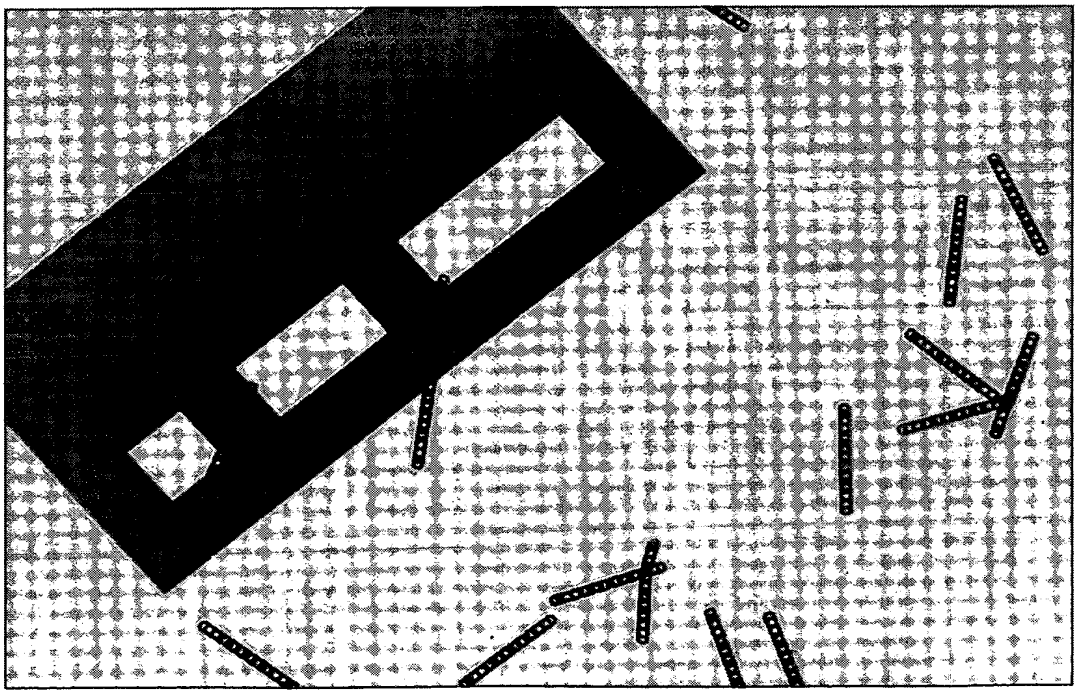

Figure 3

7. Assuming a minimum requirement, for statistical significance and data generation, to read five particles of each barcode type per assay test, then only two parallel tests (i.e. in which particles with one barcode type carry a first sample analyte and particles with another barcode type carry a second sample analyte) could be simultaneously performed with the large particles of Figure 1. On the other hand, we typically find that 600 to 800 small particles of the type shown in Figures 2 and 3 are sufficiently isolated from each other in a 7 mm diameter well to be identifiable and readable. With the same minimum requirement to read five particles of each barcode type, this provides a capability simultaneously to perform between 120 and 160 parallel assay tests (although, in practice, fewer tests with more particles per test are usually performed in order to provide more robust statistics and increase the number of data points per test).

8. Of course, the "Aurenius" particles are even larger than the 0.3 x 0.6 mm large particles of Figure 1, which further reduces their capability to be used to perform simultaneous parallel assay tests in standard wells. Furthermore, most existing laboratory liquid handling systems have tips and pipettes with bore-hole sizes of around 0.5 mm. This means that specialist measures, involving inconvenience and expense, would need to be taken to handle "Aurenius" particles. Particles of this size would also be subject to greater stresses and be more easily damaged and or distorted.

9. In addition, and even ignoring the increased manufacturing cost of larger particles themselves, "Aurenius" particles have over a 1000 times greater surface area than the 0.1 x 0.01 x 0.001 mm sized particles of Figures 2 and 3 which embody the claimed support. This three orders of magnitude difference would translate directly into the quantities of sample analyte required to coat the particle surface. In practice, a requirement for such large quantities would be unacceptable where sample analytes are limited in availability or are expensive.

10. To summarise, the images of Figures 1 to 3 demonstrate that particles embodying the claimed support would provide greatly superior results over "Aurenius" particles if used to perform assay tests in the 7 mm diameter wells of a standard microtiter plate. The superior results follow directly from being able to provide a much greater number of physically isolated particles per unit area. This dramatically increases the robustness of the statistics and the number of data points for each test performed, and allows large numbers of parallel tests to be performed simultaneously in each well. Indeed, it is doubtful whether "Aurenius" particles could be used to perform simultaneous parallel tests in each well at all, as there would probably be an insufficient number of physically isolated "Aurenius" particles available for statistical significance and data generation.

11. I further declare that all statements made herein of my knowledge are true, and that all statements made on information and belief, including those that can be supported by citations to published scientific literature, are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the '195 application or any patent issued thereon.

21-6-06
DATE

PETER SWARBRICK, Ph.D.